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(71) Applicant (for all designated States except US): SAINT
LOUIS UNIVERSITY [US/US]; Saint Louis University
Technology, Transfer Office, 3556 Caroline Mall, Suite
C110, Saint Louis, MO 63104 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): YACIUK, Peter
[US/US]; 130 S. Gore Street, Saint Louis, MO 63119
(US).

(74) Agent: ZAHNER, Joseph; Saint Louis University, 3556
Caroline Mall, Suite C110, Saint Louis, MO 63104 (US).

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(54) Title: KITS AND METHODS FOR MAKING LARGE RECOMBINANT POLYNUCLEOTIDES

(57) Abstract: Cloning of manipulated polynucleotide sequences is time consuming, labor intensive and inefficient, especially when polynucleotide sequences are long. Disclosed are methods and kits for manipulating and cloning large polynucleotide sequences that are both efficient and easy to use relative to previously described methods and kits. The disclosed invention utilizes polynucleotide fragments with mutually non-complementary asymmetric single-stranded sticky ends, which may be manipulated to produced desired sequences, then allowed to reassemble in vitro into a useful recombinant polynucleotide. Additionally, an excess of synthetic double-stranded oligonucleotides or PCR generated polynucleotides, which contain single-stranded sticky ends that are compatible with the sticky ends of the polynucleotide fragments derived from a parent polynucleotide, may be added to the polynucleotide fragments to compete with those polynucleotide fragments for ligation into a full-length recombinant polynucleotide product.



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